The impact of vitamin D deficiency on diabetes and cardiovascular risk

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Purpose of review

To review the association between vitamin D deficiency and diabetes and cardiovascular risk.

Recent findings

Vitamin D deficiency is newly recognized as a common condition of increasing prevalence worldwide. Clinically, vitamin D has an established role in calcium and bone metabolism and has recently been shown to be associated with increased risk of developing type 1 and type 2 diabetes mellitus and cardiovascular disease (CVD), as well as with cardiovascular risk factors such as hypertension and obesity. The molecular mechanisms of these associations remain incompletely understood. The active metabolite of vitamin D regulates transcription of multiple gene products with antiproliferative, prodifferentiative, and immunomodulatory effects. Although vitamin D deficiency is frequently unrecognized clinically, laboratory measurement is easy to perform and treatment of vitamin D deficiency is relatively well tolerated and inexpensive. Limited, yet promising, results of proof-of-concept intervention studies of using vitamin D in diabetes will be presented.

Summary

The high prevalence of vitamin D deficiency and plausible molecular mechanisms linking this to diabetes and cardiovascular risk suggest treatment of vitamin D deficiency to prevent and/or treat diabetes is a promising field to explore.

Keywords

cardiovascular risk/disease, diabetes, vitamin D

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Introduction

Vitamin D deficiency is common in all age groups worldwide [1–4,5•], with a dramatic increase in prevalence and severity over the last decade. The prevalence of vitamin D deficiency (<75 nmol/l) is 74%. The prevalence of severe deficiency (<25 nmol/l) increased from 2 to 6% in the general USA population and from 9 to 29% in non-Hispanic blacks, exemplified by the National Health and Nutrition Examination Survey (NHANES) report [6•]. A meta-analysis of 394 studies performed worldwide challenges traditional concepts that vitamin D is inversely associated with latitude [5•].

Type 2 diabetes mellitus (T2DM) affects morbidity and mortality [7,8], with cardiovascular disease (CVD) being the leading cause of death [9]. Vitamin D deficiency has been associated with T2DM and other established cardiovascular risk factors including hypertension, hyperlipidemia, and obesity [10]. Low vitamin D (<50 nmol/l) is independently associated with incident CVD [11], heart failure, and peripheral artery disease prevalence [12]. Most studies suggesting a link between vitamin D deficiency, T2DM, and cardiovascular risk are epidemiologic or cross-sectional in nature. Limited intervention studies have been conducted. However, as vitamin D deficiency is common [1–4,5•,6•] and potentially easy to treat, it is an attractive intervention target for further investigation in an attempt to prevent or even treat T2DM or cardiovascular disorders.

Vitamin D

Dietary sources of vitamin D are limited (Table 1). Ultraviolet B (UVB) light-dependent conversion of 7-dehydrocholesterol to cholecalciferol (vitamin D_3) in the skin is the main source vitamin D. Vitamin D_3 is hydroxylated in the liver by the vitamin D_3 25-hydroxylase to 25-hydroxycholecalciferol [25(OH)D], which is further processed in the kidney by the 25-hydroxyvitamin D_3-1α-hydroxylase to the biologically active metabolite 1,25-dihydroxycholecalciferol [1,25$(OH)_2$D]. 1,25$(OH)_2$D is a steroid hormone that regulates transcription of over 200 genes [3]. Classically known for its role in calcium and bone metabolism, vitamin D is newly

Table 1 Nutritional sources of vitamin D

Food	Serving size	International units (IU) per serving
Cod liver oil	1 Tablespoon	1360
Salmon, cooked	100 g (3.5 oz)	934
Mackerel, cooked	100 g (3.5 oz)	457
Sardines, canned in oil	50 g (1.75 oz) (drained)	96
Tuna fish, canned in oil	85 g (3 oz)	180
Milk, nonfat, reduced fat, and whole, vitamin D fortified	1 Cup	98
Margarine, fortified	1 Tablespoon	60
Pudding, prepared from mix and made with vitamin D fortified milk	Half cup	50
Ready-to-eat cereals fortified with 10% of the DV for vitamin D	Three-quarter cup to one cup servings (servings vary according to the brand)	40
Egg (vitamin D is found in egg yolk)	1 Whole medium	20
Liver, beef, cooked	100 g (3.5 oz)	46
Cheese, Swiss	30 g (1 oz)	6

recognized for potent antiproliferative, prodifferentiative, and immunomodulatory effects in many tissues [13]. The nuclear vitamin D receptor is present in most human tissues [14] and 25-hydroxyvitamin D_3-1α-hydroxylase is expressed in multiple tissues other than the kidney [15], providing potential molecular and cellular mechanisms for diverse effects of vitamin D.

Vitamin D adequacy is evaluated by measuring serum 25(OH)D concentration, as this is the primary circulating form of vitamin D. Historically, 25(OH)D concentrations required to prevent osteomalacia and rickets (>25 nmol/l) were considered normal. However, epidemiological studies suggest higher levels are necessary to prevent osteoporosis, fractures, colon cancer, and periodontal disease and improve lower extremity function [16]. Therefore, a cutoff level of 75 nmol/l is presently recommended to define vitamin D adequacy [17]. As the most commonly evaluated adult supplement dose of 400 IU daily may be insufficient when considering achieved serum vitamin D concentrations [18] or disease prevention [19,20], results of intervention studies with vitamin D should be cautiously interpreted. Daily intake of 800–1000 IU is more optimal for maintenance of normal vitamin D concentrations, and substantially larger doses are needed to treat vitamin D deficiency [3,17].

Vitamin D and diabetes

Vitamin D deficiency is associated with both type 1 diabetes mellitus (T1DM) and T2DM.

Type 1 diabetes mellitus

The immunomodulatory effects of vitamin D [13] suggest a plausible role in autoimmune diseases, such as T1DM. Indeed, lifelong treatment with 1,25$(OH)_2$D prevents insulitis and diabetes in nonobese diabetic (NOD) mice [21]. Plasma 25(OH)D concentrations are lower in patients with T1DM at diagnosis than in nondiabetic controls (82.5 vs. 96.7 nmol/l; $P < 0.0001$) [22], although mean levels for both groups were in the normative range. Importantly, vitamin D supplementation in infancy decreased incident T1DM demonstrated in a large European multicenter cross-sectional trial [23] and a Finish birth-cohort study. In the latter study, children who regularly took 2000 IU daily during the first year of life had a relative risk (RR) of 0.22 [95% confidence interval (CI) 0.05–0.89] for developing T1DM by age 30 compared with those who used less. Children with suspected rickets had a RR of 3.0 (95% CI 1.0–9.0) compared with those without such suspicion [24]. No study thus far has assessed in a prospective randomized manner the effect of vitamin D on T1DM prevention. In recent-onset T1DM, administration of 0.25 μg calcitriol [1,25$(OH)_2$D] on alternate days for 1 year had a modest effect on residual pancreatic beta-cell function and temporarily reduced insulin requirement at 3 and 6 months [25], suggesting a potential therapeutic role after diagnosis.

Type 2 diabetes mellitus

The pathogenesis of T2DM involves both beta-cell dysfunction and insulin resistance. In-vitro and rodent studies suggest an important role for vitamin D both in beta-cell function and insulin resistance [26]. Hypovitaminosis D correlates with beta-cell dysfunction and insulin resistance in 126 normoglycemic healthy adults studied with an oral glucose tolerance test (OGTT) and hyperglycemic clamp [27].

Multiple epidemiologic studies suggest low vitamin D levels are associated with impaired glucose metabolism. 25(OH)D correlates inversely with fasting serum glucose, with the greatest association observed when 25(OH)D concentrations were less than 40 nmol/l in nondiabetic postmenopausal women [28]. Likewise, serum 25(OH)D is inversely associated with hemoglobin A1c (HbA1C) especially at concentrations less than 65 nmol/l and in participants with higher BMI [29]. Hypovitaminosis D is more prevalent in patients with T2DM than in those without [30] and is associated with increased diabetes prevalence and insulin resistance assessed by fasting

glucose and insulin levels in both non-Hispanic whites and Mexican Americans [31]. Similarly, a significant inverse association was observed between serum 25(OH)D concentration and T2DM incidence in a 17-year observational Finish study, with a RR of 0.60 (95% CI 0.36–0.98; $P_{trend} = 0.01$) between highest and lowest serum 25(OH)D quartile [32]. Lastly, baseline 25(OH)D was inversely associated with 10-year risk of hyperglycemia (fasting glucose and 2 h post-OGTT) and insulin resistance [fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR)] in a prospective population-based study of 524 middle-aged nondiabetic men and women [33].

Only a few intervention studies evaluate effects of vitamin D supplementation on glucose metabolism. High-dose calcitriol (1.5 μg daily for 7 days) did not increase insulin sensitivity measured by euglycemic clamp compared with placebo in healthy volunteers who were not vitamin D deficient at baseline [34]. However, 1-month treatment with 1332 IU cholecalciferol daily in 10 women with T2DM (of whom seven were vitamin D deficient at baseline) improved insulin sensitivity and first-phase insulin secretion, albeit with no effect on second-phase insulin secretion [35].

The most promising study to date suggesting vitamin D alters diabetes risk is a post hoc analysis from an osteoporosis intervention study in which 314 nondiabetic white adults were randomized to either 700 IU vitamin D_3 and 500 mg calcium citrate, or placebos, daily for 3 years. Treatment with vitamin D and calcium citrate demonstrated attenuated rise in fasting glucose in participants with baseline impaired fasting glucose (IFG) but not in those with normal fasting glucose. Participants with IFG who received vitamin D and calcium also had reduced progression of insulin resistance assessed by HOMA-IR [36]. Conversely, the Women's Health Initiative (WHI) study reported that supplementation with 400 IU vitamin D_3 and 1000 mg calcium daily compared with placebo did not reduce risk of developing diabetes over 7 years [37].

Considering the results of these two studies, it is important to note that neither was designed to assess diabetes or glucose metabolism as primary outcome. The diagnosis of abnormal glucose metabolism or T2DM was based solely on fasting glucose or self-reported diabetes, which are insufficient to comprehensively diagnose diabetes. Furthermore, study participants were not recruited according to T2DM risk and may not represent appropriate target population for T2DM prevention. As baseline concentrations of vitamin D were not reported, it is plausible that only people with low levels benefit from vitamin D supplementation. In addition, administered vitamin D doses, especially in the WHI, are no longer considered sufficient for disease prevention [17] and post-treatment levels of vitamin D are not reported, raising the possibility that even actively supplemented patients did not reach adequate 25(OH)D concentrations. In both studies, vitamin D was given with calcium, complicating the distinction of vitamin D effect itself. Finally, placebo-treated patients in the WHI were allowed to take vitamin D and/or calcium supplements, which may attenuate observed effect of active treatment.

A meta-analysis of observational and intervention studies suggests inadequate vitamin D and calcium intake negatively influence glycemia, whereas combined supplementation with both nutrients may be beneficial in optimizing glucose metabolism [26]. Clinical studies designed specifically to assess the effect of vitamin D supplementation on glucose metabolism are currently underway [38]. These studies evaluate the effect of vitamin D supplementation in high-risk populations with both vitamin D deficiency and impaired glucose metabolism. Larger doses of vitamin D, 2000–7000 IU daily, are used. The results of these studies will be informative and are timely.

Vitamin D and obesity

Obesity is an established risk factor for T2DM. Many studies demonstrate serum 25(OH)D concentrations are inversely correlated with various measures of obesity including weight, BMI, and waist circumference [4,28,39–41]. The proposed explanation for the relationship between low vitamin D and obesity is reduced bioavailability of vitamin D in obese people after either UVB exposure or oral administration of vitamin D because of its sequestration in adipose tissue [42]. Although obesity is associated with lower vitamin D levels, evidence of beneficial effect of vitamin D supplementation on weight reduction is lacking [43,44].

Vitamin D and the metabolic syndrome

The metabolic syndrome, describing the cluster of abdominal obesity, hypertension, dyslipidemia, and hyperglycemia [45], is closely linked with T2DM [46]. Two cross-sectional studies (NHANES III and NHANES 2003–2004) have shown a significant inverse association between serum vitamin D concentrations and the metabolic syndrome [41,47]. Low serum 25(OH)D was associated with components of the metabolic syndrome including abdominal obesity, hypertension, hypertriglyceridemia, and higher HbA1C and lower high-density cholesterol [48]. Likewise, baseline 25(OH)D levels were inversely associated with 10-year risk of metabolic syndrome risk score in a prospective longitudinal study from the UK [33], although this relationship has not been uniformly replicated [49].

Vitamin D and blood pressure

The majority of patients with T2DM also have hypertension [50]. Rodent studies demonstrate $1,25(OH)_2D$ is a negative regulator of the renin–angiotensin system, which plays a major role in blood pressure regulation [51,52].

Serum vitamin D concentrations inversely correlate with blood pressure both in normotensive and hypertensive individuals in cross-sectional studies, even in the normative range of serum vitamin D [53,54]. In large prospective cohort studies, low levels of 25(OH)D (<37.5 nmol/l) compared with normal levels (>75 nmol/l) were associated with 6.1-fold and 2.7-fold increased relative risk (RR) of developing hypertension, in men and women, respectively, over 4–8 years [55].

Eight weeks intervention with 800 IU vitamin D3 plus 1200 mg calcium daily in elderly women with low baseline 25(OH)D concentrations (<50 nmol/l) led to a significant reduction in SBP and heart rate compared with calcium alone. Although this was a relatively small study, 81% of the vitamin D–calcium group had a 5 mmHg or more decline in SBP, compared with 47% of patients in the calcium-alone group [56].

In contrast, 400 IU vitamin D_3 plus 1000 mg elemental calcium daily did not affect the change over time in SBP or DBP in the WHI trial in which 36 282 postmenopausal women were followed for a median of 7 years [57]. These null results should be cautiously interpreted, as again, the WHI was not specifically designed to assess the effect of vitamin D on blood pressure, the vitamin D supplement dose was significantly lower than the dose that may be necessary and patients in the placebo group were allowed to take vitamin D supplement, which may have attenuated observed findings.

Vitamin D and cardiovascular disease

Vitamin D deficiency is independently associated with cardiovascular risk. In a case–control study of 179 patients with prior myocardial infarction (MI) and 179 people with no ischemic heart disease matched by age, sex, and date of blood collection (important for seasonal variation in vitamin D concentrations), MI patients had significantly lower mean 25(OH)D concentrations (32.0 versus 35.5 nmol/l; $P = 0.017$), although both groups manifest low vitamin D levels [58].

Vitamin D deficiency increases the risk of prevalent CVD (odds ratio 1.20) after adjustment for multiple risk factors in cross-sectional analysis from the NHANES III [59]. A Health Professionals Follow-up Study conducted in 18 225 middle-aged men free of diagnosed CVD at baseline [60] showed that men deficient in 25(OH)D ($\leq$37.5 nmol/l) have increased risk for MI compared with those sufficient ($\geq$75 nmol/l) in vitamin D (RR 2.42, 95% CI 1.53–3.84; $P < 0.001$ for trend) [60]. Similarly, there were two-fold increases in all-cause mortality and 2.2-fold increases in cardiovascular mortality risk in patients in the lower two quartiles of 25(OH)D (median, 19.0 and 32.3 nmol/l) compared with the highest quartile (median, 71 nmol/l) in a prospective Austrian study [61].

Coronary calcification measured by computed tomography correlates with coronary artery disease (CAD) severity and predicts CAD both in symptomatic and asymptomatic individuals [62,63]. Coronary calcification negatively correlated with 1,25(OH)D concentrations both in asymptomatic people with moderate risk of developing CVD and in very high risk patients homozygous for familial hypercholesterolemia with known CVD [64]. Unfortunately, this study reports concentrations of 1,25(OH)D, which do not correlate well with 25(OH)D, and may not represent a good measure of vitamin D body stores [65].

Vitamin D and calcium supplementation have not been shown to alter either coronary or cerebrovascular risk over a 7-year period in the WHI study [66]. Although CVD was a prespecified secondary efficacy outcome in the WHI, the null results regarding vitamin D effect on cardiovascular risk should be cautiously interpreted for similar reasons as T2DM risk in the WHI discussed above.

In addition to effect on cardiovascular risk factors, vitamin D may impact cardiac function and atherosclerosis through multiple other mechanisms. Increased activity of the renin–angiotensin–aldosterone system has an important role in cardiovascular risk beyond its effect on blood pressure [67,68] and vitamin D is a negative regulator of the renin–angiotensin system [51,52]. In addition, vitamin D deficiency affects cardiac contractility, vascular tone, cardiac collagen content, and cardiac tissue maturation [69] and has direct effects on vascular smooth muscle cell calcification and proliferation [70–73]. Chronic subacute inflammation had been shown to increase cardiovascular risk [74]. As a modulator of inflammatory cells and inflammatory cytokines secretion [75], low vitamin D has been implicated to contribute to several chronic inflammatory conditions [13]. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a key transcription factor regulating the expression of multiple proinflammatory and proatherogenic cytokines. Increased NF-κB activity has been associated with insulin resistance, T2DM, and atherosclerosis [76]. Vitamin D has been suggested to inhibit NF-κB activity [77,78] and blunt lipopolysaccharide-induced mRNA expressions of proatherogenic factors [79]. In addition, $1,25(OH)_2D_3$ may suppress macrophage

foam cell formation in diabetic patients by reducing acetylated or oxidized low-density lipoprotein (LDL) cholesterol uptake. Conversely, deletion of the macrophage vitamin D receptor accelerates foam cell formation induced by modified LDL. $1,25(OH)_2D_3$ has been demonstrated to reduce c-Jun N-terminal kinase (JNK) activation, peroxisome proliferated–activated receptor-gamma (PPARγ), the thrombospondin receptor CD36, and scavenger receptor-A1 (SR-A1) expression while improving endoplasmic reticulum stress and insulin signaling [80]. Together, these cellular and molecular mechanisms may underlie the physiologic contribution of vitamin D to diabetes and cardiovascular risk.

Management of vitamin D deficiency

Presently, there are no recommendations to screen for vitamin D deficiency. With many known risk factors (listed below), millions of people worldwide are at high risk for vitamin D deficiency making screening impractical and economically challenging.

(1) Lack/reduced sun exposure:
   (a) institutionalized;
   (b) living in high or low latitude;
   (c) avoidance of unprotected sun exposure or spending most day indoor; and
   (d) wearing clothes that reduce UVB exposure (i.e. because of religious beliefs).
(2) Nutritional/absorptive:
   (a) malnutrition, decreased nutritional vitamin D intake;
   (b) small bowel resection;
   (c) gastric surgery (resection or gastric bypass);
   (d) total parenteral nutrition (TPN);
   (e) hepatic disease – reduced vitamin D absorption; and
   (f) breast-fed infants: vitamin D requirements cannot be met by human milk alone.
(3) Reduced vitamin D synthesis:
   (a) elderly – less efficient synthesis of vitamin D in the skin;
   (b) dark skin (people with darker skin require three-fold to six-fold more exposure);
   (c) hepatic disease – decreased 25(OH)D synthesis; and
   (d) renal disease – decreased $1,25(OH)_2D$ synthesis.
(4) Reduced bioavailability:
   (a) obesity.
(5) Increased requirement:
   (a) pregnancy.
(6) Medications that accelerate conversion of vitamin D to inactive metabolites:
   (a) anticonvulsant drugs (requires tow-fold to five-fold more vitamin D intake daily) and
   (b) rifampin.
(7) Medications that interfere with absorption:
   (a) bile acid sequestrants and
   (b) laxatives.

However, this must be balanced against reports of rising vitamin D deficiency prevalence and the many health-related conditions associated with it, suggesting the need for appropriate vitamin D supplementation. Current vitamin D daily recommended intake (DRI) of 200 IU from birth through age 50 years, 400 IU from age 51 to 70, and 600 IU from above 70 years is likely insufficient to achieve optimal vitamin D level of 75 nmol/l [18]. Vitamin D intoxication, which can be serious, causing hypercalcemia and/or hypercalciuria [81], is rare and may be caused by inadvertent or intentional ingestion of excessively high doses becoming apparent when 25(OH)D levels exceed 250–375 nmol/l [82,83]. For example, 5000 IU vitamin D daily supplement for 12 months in sun-deprived nursing home residents did not change serum calcium or caused hypercalcemia [84]. The upper limit of vitamin D intake may be as high as 10 000 IU daily [85]. Therefore, established safety profile of vitamin D supplementation provides a wide safety margin and supports an adult supplement dose of 800–1000 IU daily [3,17]. People with overt vitamin D deficiency require 10 000–50 000 IU vitamin D monthly until stores are replete. On average, 100 IU of vitamin D increases 25(OH)D serum concentration by 2–3 nmol/l [17]. Importantly, due to accumulating information about the apparent need to change the DRI of vitamin D, a committee of the Institute of Medicine has been named to assess current relevant data and update the DRIs [86]. The committee's conclusions are expected to be presented in late spring, 2010.

Conclusion

The importance of vitamin D to bone and skeletal muscle health is well established. However, evidence is accumulating to suggest vitamin D plays an important role in other conditions such as diabetes and cardiovascular health, as well as cancer, and autoimmune and dental diseases [3,16]. It is also evident that vitamin D deficiency or insufficiency is extremely prevalent worldwide, with apparent increasing prevalence. Currently, there is insufficient level 1 evidence from intervention studies evaluating whether vitamin D supplementation could improve diabetes and cardiovascular risk or outcomes. However, given the ease and safety to administer vitamin D, recommending adequate vitamin D intake should be considered in appropriate patients while awaiting sounder evidence from prospective, randomized intervention studies.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 177–178).

1 Orwoll E, Nielson CM, Marshall LM, *et al.* Vitamin D deficiency in older men. J Clin Endocrinol Metab 2009; 94:1214–1222.

2 Kumar J, Muntner P, Kaskel FJ, *et al.* Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001-2004. Pediatrics 2009; 124:e362–e370.

3 Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357:266–281.

4 Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. Am J Clin Nutr 2007; 85:860–868.

5 • Hagenau T, Vest R, Gissel TN, *et al.* Global vitamin D levels in relation to age, gender, skin pigmentation and latitude: an ecologic meta-regression analysis. Osteoporos Int 2009; 20:133–140.

A recent meta-analysis of multiple cross-sectional studies of vitamin D status globally.

6 • Ginde AA, Liu MC, Camargo CA Jr. Demographic differences and trends of vitamin D insufficiency in the US population, 1988–2004. Arch Intern Med 2009; 169:626–632.

A recent report of increased vitamin D deficiency prevalence in the USA.

7 Bertoni AG, Krop JS, Anderson GF, Brancati FL. Diabetes-related morbidity and mortality in a national sample of U.S. elders. Diabetes Care 2002; 25:471–475.

8 Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature 2001; 414:782–787.

9 Fang J, Alderman MH. Impact of the increasing burden of diabetes on acute myocardial infarction in New York City: 1990–2000. Diabetes 2006; 55: 768–773.

10 Martins D, Wolf M, Pan D, *et al.* Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. Arch Intern Med 2007; 167:1159–1165.

11 Wang TJ, Pencina MJ, Booth SL, *et al.* Vitamin D deficiency and risk of cardiovascular disease. Circulation 2008; 117:503–511.

12 Kim DH, Sabour S, Sagar UN, *et al.* Prevalence of hypovitaminosis D in cardiovascular diseases (from the National Health and Nutrition Examination Survey 2001 to 2004). Am J Cardiol 2008; 102:1540–1544.

13 Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. Endocr Rev 2005; 26:662–687.

14 Norman AW. Minireview: vitamin D receptor: new assignments for an already busy receptor. Endocrinology 2006; 147:5542–5548.

15 Townsend K, Evans KN, Campbell MJ, *et al.* Biological actions of extra-renal 25-hydroxyvitamin D-1alpha-hydroxylase and implications for chemoprevention and treatment. J Steroid Biochem Mol Biol 2005; 97:103–109.

16 Bischoff-Ferrari HA, Giovannucci E, Willett WC, *et al.* Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006; 84:18–28.

17 Vieth R, Bischoff-Ferrari H, Boucher BJ, *et al.* The urgent need to recommend an intake of vitamin D that is effective. Am J Clin Nutr 2007; 85:649–650.

18 Holick MF, Siris ES, Binkley N, *et al.* Prevalence of Vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab 2005; 90:3215–3224.

19 Bischoff-Ferrari HA, Willett WC, Wong JB, *et al.* Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA 2005; 293:2257–2264.

20 Broe KE, Chen TC, Weinberg J, *et al.* A higher dose of vitamin d reduces the risk of falls in nursing home residents: a randomized, multiple-dose study. J Am Geriatr Soc 2007; 55:234–239.

21 Mathieu C, Waer M, Laureys J, *et al.* Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3. Diabetologia 1994; 37:552–558.

22 Littorin B, Blom P, Scholin A, *et al.* Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). Diabetologia 2006; 49:2847–2852.

23 Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. The EURODIAB Substudy 2 Study Group. Diabetologia 1999; 42:51–54.

24 Hypponen E, Laara E, Reunanen A, *et al.* Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 2001; 358:1500–1503.

25 Pitocco D, Crino A, Di Stasio E, *et al.* The effects of calcitriol and nicotinamide on residual pancreatic beta-cell function in patients with recent-onset Type 1 diabetes (IMDIAB XI). Diabet Med 2006; 23:920–923.

26 Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab 2007; 92:2017–2029.

27 Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr 2004; 79:820–825.

28 Need AG, O'Loughlin PD, Horowitz M, Nordin BE. Relationship between fasting serum glucose, age, body mass index and serum 25 hydroxyvitamin D in postmenopausal women. Clin Endocrinol (Oxf) 2005; 62:738–741.

29 Hypponen E, Power C. Vitamin D status and glucose homeostasis in the 1958 British birth cohort: the role of obesity. Diabetes Care 2006; 29:2244–2246.

30 Targher G, Bertolini L, Padovani R, *et al.* Serum 25-hydroxyvitamin D3 concentrations and carotid artery intima-media thickness among type 2 diabetic patients. Clin Endocrinol (Oxf) 2006; 65:593–597.

31 Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care 2004; 27:2813–2818.

32 Mattila C, Knekt P, Mannisto S, *et al.* Serum 25-hydroxyvitamin D concentration and subsequent risk of type 2 diabetes. Diabetes Care 2007; 30:2569–2570.

33 Forouhi NG, Luan J, Cooper A, *et al.* Baseline serum 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. Diabetes 2008; 57:2619–2625.

34 Fliser D, Stefanski A, Franek E, *et al.* No effect of calcitriol on insulin-mediated glucose uptake in healthy subjects. Eur J Clin Invest 1997; 27:629–633.

35 Borissova AM, Tankova T, Kirilov G, *et al.* The effect of vitamin D3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. Int J Clin Pract 2003; 57:258–261.

36 Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care 2007; 30:980–986.

37 de Boer IH, Tinker LF, Connelly S, *et al.* Calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative. Diabetes Care 2008; 31:701–707.

38 NIH, National Institute of Health. http://clinicaltrials.gov/.

39 Macdonald HM, Mavroeidi A, Barr RJ, *et al.* Vitamin D status in postmenopausal women living at higher latitudes in the UK in relation to bone health, overweight, sunlight exposure and dietary vitamin D. Bone 2008; 42:996–1003.

40 Konradsen S, Ag H, Lindberg F, *et al.* Serum 1,25-dihydroxy vitamin D is inversely associated with body mass index. Eur J Nutr 2008; 47:87–91.

41 Ford ES, Ajani UA, McGuire LC, Liu S. Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. Diabetes Care 2005; 28:1228–1230.

42 Wortsman J, Matsuoka LY, Chen TC, *et al.* Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000; 72:690–693.

43 Sneve M, Figenschau Y, Jorde R. Supplementation with cholecalciferol does not result in weight reduction in overweight and obese subjects. Eur J Endocrinol 2008; 159:675–684.

44 Major GC, Alarie FP, Dore J, Tremblay A. Calcium plus vitamin D supplementation and fat mass loss in female very low-calcium consumers: potential link with a calcium-specific appetite control. Br J Nutr 2009; 101:659–663.

45 Grundy SM, Brewer HB Jr, Cleeman JI, *et al.* Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004; 109:433–438.

46 Ford ES, Li C, Sattar N. Metabolic syndrome and incident diabetes: current state of the evidence. Diabetes Care 2008; 31:1898–1904.

47 Reis JP, von Muhlen D, Miller ER 3rd. Relation of 25-hydroxyvitamin D and parathyroid hormone levels with metabolic syndrome among US adults. Eur J Endocrinol 2008; 159:41–48.

48 Hypponen E, Boucher BJ, Berry DJ, Power C. 25-Hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. Diabetes 2008; 57:298–305.

49 McGill AT, Stewart JM, Lithander FE, *et al.* Relationships of low serum vitamin D3 with anthropometry and markers of the metabolic syndrome and diabetes in overweight and obesity. Nutr J 2008; 7:4.

50 Kabakov E, Norymberg C, Osher E, *et al.* Prevalence of hypertension in type 2 diabetes mellitus: impact of the tightening definition of high blood pressure and association with confounding risk factors. J Cardiometab Syndr 2006; 1:95–101.

51 Li YC, Kong J, Wei M, *et al.* 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest 2002; 110:229–238.

52 Zhou C, Lu F, Cao K, *et al.* Calcium-independent and 1,25(OH)2D3-dependent regulation of the rennin–angiotensin system in 1alpha-hydroxylase knockout mice. Kidney Int 2008; 74:170–179.

53 Kristal-Boneh E, Froom P, Harari G, Ribak J. Association of calcitriol and blood pressure in normotensive men. Hypertension 1997; 30:1289–1294.

54 Lind L, Hanni A, Lithell H, *et al.* Vitamin D is related to blood pressure and other cardiovascular risk factors in middle-aged men. Am J Hypertens 1995; 8:894–901.

55 Forman JP, Giovannucci E, Holmes MD, *et al.* Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension 2007; 49:1063–1069.

56 Pfeifer M, Begerow B, Minne HW, *et al.* Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. J Clin Endocrinol Metab 2001; 86:1633–1637.

57 Margolis KL, Ray RM, Van Horn L, *et al.* Effect of calcium and vitamin D supplementation on blood pressure: the Women's Health Initiative Randomized Trial. Hypertension 2008; 52:847–855.

58 Scragg R, Jackson R, Holdaway IM, *et al.* Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: a community-based study. Int J Epidemiol 1990; 19:559–563.

59 Kendrick J, Targher G, Smits G, Chonchol M. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. Atherosclerosis 2009; 205:255–260.

60 Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-Hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. Arch Intern Med 2008; 168:1174–1180.

61 Dobnig H, Pilz S, Scharnagl H, *et al.* Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. Arch Intern Med 2008; 168:1340–1349.

62 Thompson BH, Stanford W. Update on using coronary calcium screening by computed tomography to measure risk for coronary heart disease. Int J Cardiovasc Imaging 2005; 21:39–53.

63 Dendukuri N, Chiu K, Brophy JM. Validity of electron beam computed tomography for coronary artery disease: asystematic review and meta-analysis. BMC Med 2007; 5:35.

64 Watson KE, Abrolat ML, Malone LL, *et al.* Active serum vitamin D levels are inversely correlated with coronary calcification. Circulation 1997; 96:1755–1760.

65 Need AG, Horowitz M, Morris HA, Nordin BC. Vitamin D status: effects on parathyroid hormone and 1, 25-dihydroxyvitamin D in postmenopausal women. Am J Clin Nutr 2000; 71:1577–1581.

66 Hsia J, Heiss G, Ren H, *et al.* Calcium/vitamin D supplementation and cardiovascular events. Circulation 2007; 115:846–854.

67 Farmer JA. Renin angiotensin system and ASCVD. Curr Opin Cardiol 2000; 15:141–150.

68 Milliez P, Girerd X, Plouin PF, *et al.* Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. J Am Coll Cardiol 2005; 45:1243–1248.

69 Achinger SG, Ayus JC. The role of vitamin D in left ventricular hypertrophy and cardiac function. Kidney Int Suppl 2005; (95):S37–S42.

70 Merke J, Hofmann W, Goldschmidt D, Ritz E. Demonstration of 1,25(OH)2 vitamin D3 receptors and actions in vascular smooth muscle cells in vitro. Calcif Tissue Int 1987; 41:112–114.

71 Cardus A, Parisi E, Gallego C, *et al.* 1,25-Dihydroxyvitamin D3 stimulates vascular smooth muscle cell proliferation through a VEGF-mediated pathway. Kidney Int 2006; 69:1377–1384.

72 Carthy EP, Yamashita W, Hsu A, Ooi BS. 1,25-Dihydroxyvitamin D3 and rat vascular smooth muscle cell growth. Hypertension 1989; 13 (6 Pt 2):954–959.

73 Somjen D, Weisman Y, Kohen F, *et al.* 25-Hydroxyvitamin D3-1alpha-hydroxylase is expressed in human vascular smooth muscle cells and is upregulated by parathyroid hormone and estrogenic compounds. Circulation 2005; 111:1666–16671.

74 Koenig W, Sund M, Frohlich M, *et al.* C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 1999; 99:237–242.

75 Amento EP. Vitamin D and the immune system. Steroids 1987; 49:55–72.

76 Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006; 116:1793–1801.

77 Cohen-Lahav M, Douvdevani A, Chaimovitz C, Shany S. The anti-inflammatory activity of 1,25-dihydroxyvitamin D3 in macrophages. J Steroid Biochem Mol Biol 2007; 103:558–562.

78 Cohen-Lahav M, Shany S, Tobvin D, *et al.* Vitamin D decreases NFkappaB activity by increasing IkappaBalpha levels. Nephrol Dial Transplant 2006; 21:889–897.

79 Talmor Y, Bernheim J, Klein O, *et al.* Calcitriol blunts pro-atherosclerotic parameters through NFkappaB and p38 in vitro. Eur J Clin Invest 2008; 38:548–554.

80 Oh J, Weng S, Felton SK, *et al.* 1,25(OH)2 vitamin D inhibits foam cell formation and suppresses macrophage cholesterol uptake in patients with type 2 diabetes mellitus. Circulation 2009; 120:687–698.

81 Jacobus CH, Holick MF, Shao Q, *et al.* Hypervitaminosis D associated with drinking milk. N Engl J Med 1992; 326:1173–1177.

82 Koutkia P, Chen TC, Holick MF. Vitamin D intoxication associated with an over-the-counter supplement. N Engl J Med 2001; 345:66–67.

83 Klontz KC, Acheson DW. Dietary supplement-induced vitamin D intoxication. N Engl J Med 2007; 357:308–309.

84 Mocanu V, Stitt PA, Costan AR, *et al.* Long-term effects of giving nursing home residents bread fortified with 125 microg (5000 IU) vitamin D(3) per daily serving. Am J Clin Nutr 2009; 89:1132–1137.

85 Hathcock JN, Shao A, Vieth R, Heaney R. Risk assessment for vitamin D. Am J Clin Nutr 2007; 85:6–18.

86 Institute of Medicine, Dietary Reference Intakes for Vitamin D and Calcium. http://www.iom.edu/Activities/Nutrition/DRIVitDCalcium.aspx.